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**Applicant:** Commonwealth Scientific and Industrial Research  
Organisation, The Australian National University,  
Agriculture Protection Board of Western Australia,  
Department of Conservation and Land Management

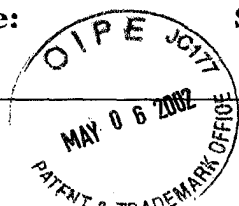
**Serial Number:** 09/530772

**Filed:**

6 November 1998

**Title:**

Suicide expression vector for use in vaccine strains

**DECLARATION UNDER 37 C.F.R. 1.132**

I, **Robert Seymour of 1 Arabilia Close, Chapel Hill, QLD, AUSTRALIA** declare that:

1. I am one of the inventors of the subject matter of US Patent Application Serial No. 09/530772 (the present application) filed on 6 November 1998.
2. My qualifications and technical experience are set out in my Curriculum Vitae, a copy of which is attached as Annexure A.
3. The present application relates to a suicide expression vector for transiently expressing a heterologous peptide, polypeptide or protein in a selected host cell and removal of recombinant plasmid DNA from said host cell.
4. I have read the Office Action dated 30 August 2001 relating to USSN 09/530772. I note that the Examiner has cited the *Herrero et al* (1990) publication in pages 3-5 of the Office Action. The Examiner believes that *Herrero et al* (1990) discloses the features of the present invention. I believe, however, that *Herrero et al* (1990) does not disclose the invention described in the application for the reasons set out below.

**Transposases and restriction enzymes:**

5. It is my view and I believe the view of others working in my field, that a transposase is not a restriction enzyme and neither does it substitute for a restriction enzyme. A transposase has a unique and very different function to that of a restriction enzyme.
6. Transposons are discreet mobile DNA sequences in the genome, able to transport themselves from one locus to another. Simple transposons called "insertion sequences" end in short inverted terminal repeats which flank one or two open reading frames. Composite transposons carry drug resistance genes or other coding sequences in addition to the functions concerned with transposition and may be contained in a chromosome or plasmid. *Herrero et al*, describes transposons Tn5 and Tn10 that are composite transposons present on a plasmid.
7. During transposition, transposase molecules bind to the short inverted repeat sequences at either end of the transposon. These molecules then bring both ends of the transposon together to form a protein/DNA complex and the transposon (simple or composite) is cleaved from the chromosome or plasmid. The nucleoprotein complex then binds to the target DNA of the host cell, usually in a random manner, and the transposase splices the transposon into the target site of the host DNA (for general

information on transposons and transposases see; Lewin B. **Genes VII** Chapter 15 p457 – 479, Haren L. *et al* **Integrating DNA: Transposases and Retroviral Integrases** *Annu. Rev. Microbiol.* 1999 53:245-81, Craig N.L. **Target site selection in transposition** *Annu. Rev. Biochem* 1997 66:437-74 and Reznikoff W.S. **The Tn5 Transposon** *Annu. Rev. Microbiol.* 1993 47:945-63).


8. In contrast, restriction enzymes recognise a single short sequence of double stranded DNA as a target for cleavage. The restriction enzyme cuts the DNA then falls from the DNA. The restriction enzyme does not need the formation of a protein complex with other restriction enzyme molecules to cleave the DNA and neither does it facilitate ligation of the cut DNA into another segment of DNA.
9. Accordingly, there are distinct differences between restriction enzymes and transposases. These differences may be summarised as follows:
  - (i) Transposases cleave two sites (inverted terminal repeats) on the original DNA and another random site on the host DNA sequence. Restriction enzymes only require one target site to be present for cleavage.
  - (ii) Transposases need to form a complex with two or more molecules of the enzyme and their target sites to result in cleavage of the DNA. Restriction enzymes only require one molecule and one target site to result in cleavage of the DNA.
  - (iii) Transposases protect the DNA ends of the released transposon from digestion to enable integration of the transposon into a new site in the chromosome. Restriction enzymes cleave the DNA and then fall off the DNA exposing the ends of the linearised DNA to degradation.
  - (iv) Transposases have a recombinase function as they splice and ligate the transposon into an alternate site or sequence. Restriction enzymes do not have this function. In this manner transposases are similar to integrases from viruses and not restriction enzymes.
10. The present invention provides a method of transiently expressing a desired heterologous peptide. The function of the restriction enzyme is to cleave the DNA expression vector at one or more locations on the plasmid. Importantly, the host cell chromosomal DNA is not cleaved. Once the suicide expression vector has been cleaved, the whole plasmid is degraded by the natural cellular mechanisms and the heterologous peptide of interest is no longer expressed in the host cell. It is important to note that the suicide expression vector is distinct from the host chromosomal DNA, and therefore when the plasmid is totally degraded the host cell comprises only host cell DNA and no recombinant DNA.
11. Herrero *et al* discloses a plasmid containing a transposon coding for a transposase. In contrast to the action of the restriction enzyme in the present invention, the transposase cleaves the host cell chromosomal DNA as well as the donor plasmid DNA. Furthermore the transposase has recombinase activity, the function of which is to splice and ligate the transposon (Tn5 or Tn10) from the donor plasmid into the chromosome of the host cell.
12. The transposons Tn5 and Tn10 include a recombinant gene expression cassette to drive expression of a foreign gene. Once transposition has occurred expression of the foreign gene will then be from the chromosome of the host cell. The aim of the transposon

therefore is to stably insert the foreign gene into the chromosome of the host cell. Following transposition the host DNA comprises an inserted transposon, which includes the foreign gene. It is clear therefore that the donor plasmid or suicide expression vector according to Herrero *et al* is only partially degraded as the transposon is transposed and not degraded.

13. Although Herrero *et al* refers to a suicide plasmid, the suicide capabilities are related to the properties of the plasmid pGP704. This plasmid contains the R6K origin of replication, which is only active in the presence of the  $\pi$  protein of the *pir* gene. The plasmid is propagated in strains of *E.coli* carrying this gene but used for transposition in strains that are devoid of this gene and therefore where the plasmid will not replicate. The activity therefore of the transposase does not result in degradation of the recombinant DNA, rather it results in retention of the recombinant DNA as part of a transposon inserted into the chromosome of the host cell. In contrast, the suicide expression vector of the present invention is stable until the restriction enzyme cleaves the plasmid. This cleavage brings about the subsequent degradation of the suicide expression vector and the recombinant DNA that is part of that vector.
14. The function of a transposase is distinctly different to that of the restriction enzyme described in the present application. The transposon described by Herrero *et al* does not encode a restriction enzyme and would not achieve the method of the present invention.

Dated this 8<sup>th</sup> day of February

2002

  
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**Robert Seymour**

**Applicant:** Commonwealth Scientific and Industrial Research  
Organisation, The Australian National University,  
Agriculture Protection Board of Western Australia,  
Department of Conservation and Land Management

**Serial Number:** 09/530772

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#### ANNEXURE A

The attached is Annexure A referred to in paragraph 2 of the Declaration made by Robert  
Seymour on *the 8th day of February* 2002

*Robert Seymour*

# Dr Robert Seymour

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## Education

1994 University of NSW, Faculty of Medicine, Sydney NSW

### Doctorate of Philosophy (PhD)

- Title: Development of a novel recombinant immunotoxin for the treatment of leukaemia
- Molecular biology/immunology/cancer biology

1987 University of New England Armidale NSW

### Bachelor of Science with honours

- Molecular biology/genetics honours degree

## Professional experience

1988 – 1993 St Vincents Hospital, Haematology Dept. Sydney NSW

### Research Assistant/PhD student

- **1988-1989:** Examined cytokine expression in bone marrow transplant patients post bone marrow transplant.
- **1989-1993:** Development of a recombinant immunotoxin for the treatment of Leukaemia.

1993-1996 CSIRO Animal Production Sydney NSW

### Post Doctoral Scientist

- Production of recombinant growth factors.
- Developed expression and purification technology that resulted in the production of tens of milligrams of a pure growth factor.

1996-2000 CSIRO Animal Production Sydney NSW

### Research Scientist

- Development of a self restricting suicide plasmid
- Plasmid developed as part of a vaccine technology programme.
- Development of novel vaccine methods eg DNA vaccines

2001 – present CSIRO Livestock Industries

### Research Scientist

- Development of signal sequence trap technology for wool biology research

## **Publications**

K., Matias C., Guiffre A., **Seymour R.**, Cooley M., Biggs J., Munro V., and Gillis S.

In Vivo Administration of Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte-Macrophage CSF, Interleukin-1 (IL-1), and IL-4, Alone and in Combination, After Murine Haematopoietic Stem Cell Transplantation. *Blood* 77: 1376-1382, 1991

Atkinson K., **Seymour R.**, Altavilla N., Cooley M., and Biggs J.

Cytokine activity after allogeneic bone marrow transplantation. IV. Production of mRNA for IL-3 and GM-CSF by mitogen-stimulated circulating mononuclear cells. *Bone Marrow Transplantation* (1992), **9**, 175-183

Atkinson K., Vos B., Kang-Er Z., Guiffre A., **Seymour R.** and Gillis S.

Effect of in vivo administration of IL-3 and IL-6, alone and in combination with G-CSF, GM-CSF or IL-1, on haematopoiesis, graft-versus-host disease and survival after murine haematopoietic stem cell transplantation. *Cytokines and Molecular Therapy* (1995), **1**, 47-55

## **Professional memberships**

Member of the Australian Biotechnology Association (ABA)

## **Awards received**

### **Transplantation Society of Australia and New Zealand:**

- Young investigator award presented three years in a row at annual dinner
- 1989, 1990, 1991

### **Haematology Society of Australia:**

- Travel grant to attend annual scientific meeting
- 1991